Spin trapping applications to photobiology

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When a molecule in its ground state (S_0) absorbs a photon of light an electron moves rapidly (within ~10^{-15} sec.) to an excited singlet energy level (^1S). The electron can return to the S_0 state by emitting energy (fluorescence) or by passing its energy to the environment ("internal conversion"). The ^1S state is fairly short-lived (~ 10^{-9} sec.) and so photochemistry does not generally occur from this state. The electron may also move ("intersystem crossing") to a triplet energy level (^3S), whence it can return to the ground state by emitting energy (phosphorescence) or by losing energy non-radiatively via intersystem crossing. Because the ^3S state is generally long-lived (10^{-3} sec. to seconds) it is from this state that most photochemistry occurs. The reactions that occur from the triplet (or singlet) state may follow a number of different pathways depending on the chemical nature of the sensitizer, the solvent and/or the presence of other molecules. In biological, and some chemical, systems Type I and/or Type II processes may occur. Type I reactions involve the generation of free radicals (or redox chemistry). In Type II (energy transfer) reactions, electronic excitation energy is transferred from ^3S to ground state (triplet) oxygen to give singlet oxygen. Free radicals from photochemical reactions may be detected directly by EPR. However, since most free radicals generated photolytically are chemically reactive, they cannot be observed by direct EPR. For such radicals the technique of spin trapping may be employed. Spin trapping is a technique in which a short-lived reactive free radical ($R^\bullet$) combines with a diamagnetic molecule ("spin trap", ST) to form a more stable free radical ("spin adduct", $SA^\bullet$) that can be detected by EPR,

$$R^\bullet + ST \rightarrow SA^\bullet$$

Spin traps are generally nitroso compounds (MNP, DBNBS), nitrones (DMPO, DEPMPO, PBN, POBN) or *aci*-anions of nitroalkanes. In the case of the nitroso and nitrone spin traps, the product is a nitroxide, while the *aci*-anions of nitroalkanes form nitroanion radicals. The main advantage of the nitroso spin traps is that they generate rich EPR spectra upon reaction with a radical, which makes identification much easier. However, nitroso spin traps suffer from a number of disadvantages including thermal and photochemical instability of the resultant adducts and the presence of a low energy visible spectrum (λmax ~ 650nm) that may result in photolysis of the spin trap. Nitrone spin traps generally produce more stable adducts than nitroso traps and are preferable for detecting oxygen-centered radicals. However, the EPR spectra of nitrone adducts often do not permit the unequivocal identification of the parent radical. Another problem that may occur in photochemical systems is that the spin trap can act as a quencher of the excited state of the photosensitizer under investigation. If quenching is physical then the excited state photosensitizer is returned to the ground state and no chemistry takes place. However, if chemical quenching occurs then photoproducts will be formed that would otherwise not be observed in the absence of the spin trap. Examples of the EPR/spin trapping approach to photobiological studies will be taken from current research on berberine, tirapazamine and Phloxine B.